

1 agctactcag gaggctgaga cagagaatc gcttgaaccc gggaggcaga ggttgacgtg
 61 agccgagatc acgccactag actccatcca gcctgggcga aagagcaaga ctccgtctca
 121 aaaaaaaaaa tcgttacaat ttatggtgga ttactccct ctcttacct catcaagaca
 181 cagcactact ttaagcaaa gtcaatgatt gaaacgcctt tctttcctaa taaaaggag
 241 attcagtcct taagattaat aatgtagtag ttacacttga ttaaagccat cctctgctca
 301 aggagaagct ggagaaggca ttctaaggaa aaaggggcag ggttgaaact cggacgcac
 361 ccaactgagcc gagacaagat tctgctgtag tcagtctgc ctgggaatct attttcaca
 421 agttctccaa aaaatgtgat gatcaaaact aggaattagt gttctgtgtc ttaggcccta
 481 aaatcttctt gtgaattcca tttttaagggt agtcgaggtg aaccgcgtct ggtctgcaga
 541 ggatagaaaa aaggccctct gatacctcaa gttagtcca cctttaaaga aggtcggaa
 601 taaagacgca aagcctttcc cggacgtgcg gaagggaac gtccttccct atggccggaa
 661 atggaacttt aatttccctt tcccccaac cagcccgccc gagagagtga ctctcacgag
 721 agccgcgaga gtcagcttg gtcagcttg ccaatccgtg cggctcgcg gctctccct tataagccga
 781 ctgcgccggc agcgaccgg gttgcggagg gttggcctgg gaggggtggt ggccattttt
 841 tgtctaacc taactgagaa gggcgtaggc gccgtgctt tgctccccc gcgctgtttt
 901 tctcgtgac tttcagcgg tttcagcgg cggaaaagcc tcggcctgcc gcttccacc gttcattcta
 961 gagcaaaaa aaaatgtcag ctgctggccc gttcgccct cccggggacc tgcggcgggt
 1021 cgctgccc gcccgcgaac ccgccttga ggcgcggtc ggccggggc ttctccggag
 1081 gcacctactg ccaccgcga gatttggtc tgtcagccg ggtctctcg gggcgaggg
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 1381 gcacctccaa agtcggccaa aatgaatgg cagtgaaggc ggtgtgcctg gagccgttcc
 1441 tgcgtgggtt ctccgtctt ccgctttttg ttgccttta tgggtgtatt acaactagt
 1501 tcctgctctg cagattttgt tgaggttttt gcttctccca aggtagatct cgaccagtcc
 1561 cctcaacggg gtgtgggaga acagtcatct ttttttgaga gatcatttaa cattaatga
 1621 atatttaatt agaagatcta aatgaacatt ggaaattgtg ttcccttaac ggtcatcggt
 1681 ttatgccaga ggttagaagt tcttttttg aaaaattaga ccttggcgat gaccttgagc
 1741 agtaggat aacccccaca agctt

Fig. 1

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1 aagcttggac ttgacaaaga aactgcagat catctggacc ccccccccc cccatttagg
 61 ttttaacaatg taccagctat ctgacttaag caaactgtgt tcctcataga taaggcggga
 121 ctgctcatgg tcattgtgaa gttcagttgg gataaacaaa ttttaagggtg cataacaaaa
 181 aacacaaaat gttggtgttt gtttaaaaaa aactaaagaa tttctggagg caggcagtta
 241 cagaaaacat gctgatattc tgagttgcct gctagttggt gccattccac cagagtgaac
 301 acatctctgt tgaccctgat tttctgtagg tctgtctgtg tgtctgtcct ttctccagca
 361 agggctgacc ctaategggg tcccaggacc caagccttga gaaaggcagt agtatgtcat
 421 ctagttgaaa tgacacattc tctacagtgt ccaaatagaca tctttgtgct agacagaaca
 481 ttttattgga tggactatgg ctgaccactt ggcttggggg gggggggaag gggccgcaa
 541 gggcgggggt ccctcatttg cttgttatta acacttgctt gtttgtttac ttgttagtag
 601 gaatctgctc taccacgtgg gttctacatg gttccacagg ggtcacctgg tccgtttttg
 661 ttttctggga cagttttcac aaatgttgct tagactccac gttggctttg aagcctacag
 721 ctatgagcct ctgtgccagt ttatgcagta gtatctctcg ggttgctcct caccgttagt
 781 agtggtgctc ttagaaggca ccgtgatttt ttgctttcca tctctttccc ctgccatgcc
 841 ttctgtggtt ctctgccagg caccaaactg ttcagaaaact ctccagcccg gtagagaacg
 901 gtagggggaa agaactgacg tgtggaaggg atgggcaggg agaagaggca ccgaactcgg
 961 tcttaaacia aaaaaaaaaa aaaaggagca ttagaaaaaa aaacaaattt gtgacctga
 1021 actacagacc tcctgcctca gcctcctaca agctgggatt ataggctcgg gtcagctacc
 1081 cttgaaatct ttttctttct ggaactcagt acctggttgg ccatgcactc acaagagatc
 1141 cgctgcctt ctgtctctca aattctggaa ttaaagattt gcgccacttt tccccacttc
 1201 cacccecggc tgtgggagtg gactgggttg aagggtggaat tttttttttt tttttttttt
 1261 tttagtgaia aaagggggga ttggaaatat ccctactttc aactctagta tatttcagaa
 1321 accaagcctc agagatgtgc gtgctgctgt gtgtgtgtgt gtatgtgtgt gtgtctcaca
 1381 gcaagaaaca gattttatta tttatttttt atttatttat tttttgcaag tgactggcta
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 1681 agcctcaaaa acaaacgtca gcgcaggagc tccaggttcg ccgggagctc cgcggcgccg
 1741 ggccgcccag tcccgtaccc gcctacagge cgcggccggc ctgggggtctt aggactccgc
 1801 tgccgcgcgc aagagctcgc ctctgtcagc cgcggggcgc cgggggctgg ggccaggccg
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 1981 cgctgcaggg cgggcccgtc gaacctgcga cttctgggga aaggggcttc ggtgtgagac
 2041 ggtagccagc caaagggtat atatcgccct cagcggccgt cccctccac ttttgtctaa
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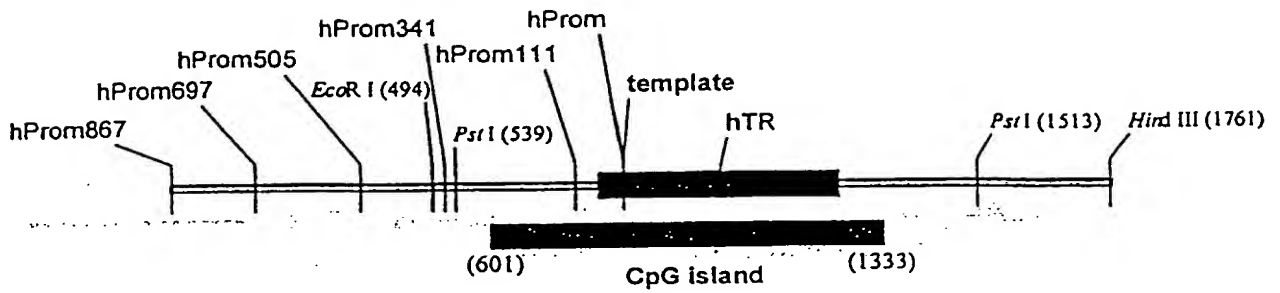
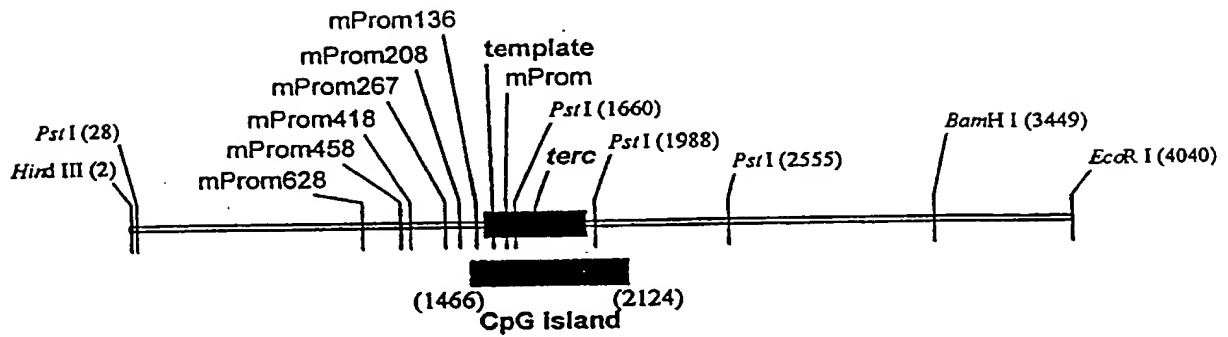
Fig. 2

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2281 ggtattccca ttgtatggga gatttttttt ttcttttgt tatgggggcg ttgaacattt
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 2401 gggatgcaaa acattacatt gaagctgaga agtttaaaga tgcgtgtctt cccctgccta
 2461 ccttcgctgt cacacagaac ctgttatctt ttcagaaaag aaaatgagat aggcagggtg
 2521 gatctggagt ttcaaggccc ttgcctgggc tgcagagtga gttaggccac accagaaaag
 2581 tatgtgtcaa aaacaaagaa gaaaggcttt gtgggggggt gggtagcaaa cgatcttaat
 2641 cccctgtgct tgggaggccc gcaaggggga tatctgtgaa ggagacaaac aaagctacac
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 2821 tgccttgact tcctcagtac ttttctgggt tttagtcata aaaaacattg aagagatgaa
 2881 gaagtgtatg tttagtaagt acataccaaa agtttgtgag ctatatgcat atagcaactc
 2941 agtcacctga aacaggcccc ttgcagctaa catatttctt agtattacta ttataaagac
 3001 taggggagtt tctaagccgg cactccttac aaggggacgaa gccatgttca gctccagctt
 3061 gccaaagatt tgaaacccaa cgtcaagcct gacgagttcg agcctggcat ctctcagccg
 3121 ctgctcgagc tggagatgac cacggatctc aaggcacagc tgtgggaact caacatcacc
 3181 gaagccaagg aaaattgaag ttggtggtgg tcagaaggct gttataattt ttgtaccagt
 3241 tcctcagctt aaatctttcc agaaaatcca agtctggcta gtttgtgaat tggagaaaaa
 3301 gttcagcggg aagcacgtgt cttcattgct cagaagagga tctgtccaag ccaaccagga
 3361 aaagctgtac gaaaaataag ccaaagcacc ctagaagctg caccctgaca gcagtgcattg
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 3481 gagcaggttc atcttcctct ctggtacatc ccatgtctcc tcatctccat cctccccctct
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 3601 ggctctagcc ttacctttca cctgccctca cctgcttata gacagcaatc tacatttctc
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 3841 atgtcctggt tcagcttgta tattagaaaa ccatctcaaa ttatatatat atatataatta
 3901 cacacacaca tatgtatata tacatatata tgtatacaca cacacacata tatatatgta
 3961 tatgtatgta tgtatgtata tatatatact tttaatgcta aatagcctgg gttggctaag
 4021 actacttcaa tcctgccaga attc

Fig. 2 (cont)

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*Fig. 3A**Fig. 3B*

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-798 hProm867
lagctactcaggaggtgtagacacgagaatcgcttgaaccgggaggcaga

-748 ggttgccagtgagccggagatcacgccactagactccatccagcctgggcga
Zeste

-698 aagagcaagactccgtctcaaaaaaaaaaatcgttacaatttatggtgga

-648 ttaactcccctctttttacctcatcaagacacagcactactttaaaagcaaaa
hProm697
GR

-598 gtcaatgattgaaacgcctttctttccataataaaagggagattcagtcct
cMYB NF1 PEA3

-548 taagattaataatgtagtagttacacttgattaaagccatcctctgctca
AFPI/BRN2

-498 aggagaagctggagaaggcattctaaggaaaaagggcagggttggaact
PEA3/c-Ets-2 Spl/NF-E2 cMYB

-448 cggaacgatccccactgagccgagacaagattctgctgtagtcagtgcgc
hProm505
Zeste GCN4/AP1

-398 ctgggaatctattttcacaaagtctccaataatgtgatgatcaaaact
myogenin GR GR

-348 aggaattagtggtctgtgtcttaggccctaaaatcttctgtgaattcca
GR/PR/AR F2F/Pit-1a Pit-1a

-298 tttttaaggtagtcgaggtgaaccggtctggtctgcagaggatagaaaa
hProm341
GATA-1

-248 aagccctctgatacctcaagttagtttcacctttaagaagggtcggaag
EIA-F

-198 taaagacgcaaagcctttccggacgtgcggaagggcaacgtccttctctc
NF1 PEA3 PU.1

-148 atggccggaatatggaactttaatttcccggttccccccaaccagcccgccc
Spl

-98 gagagagtgactctcacgagagccgcgagagtcagcttggccaatccgtg
API GCN4/AP1 CCAAT Box

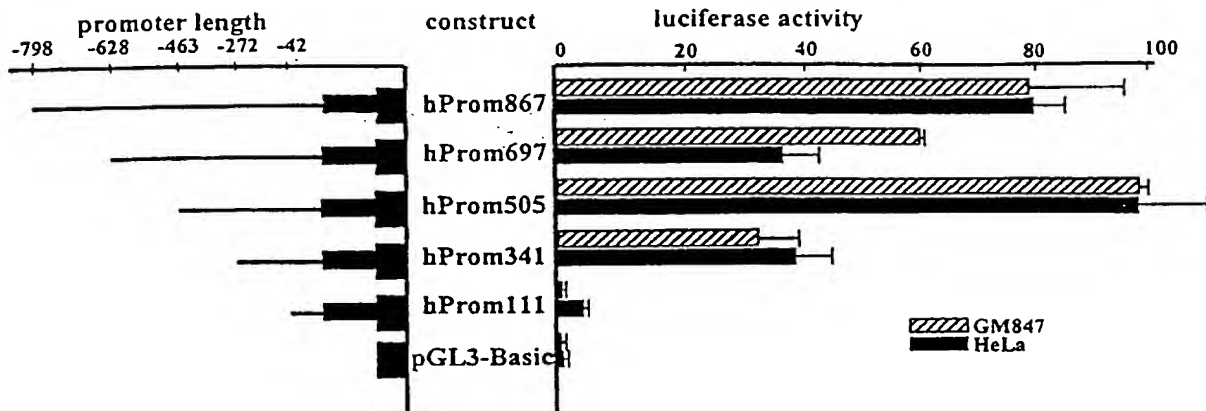
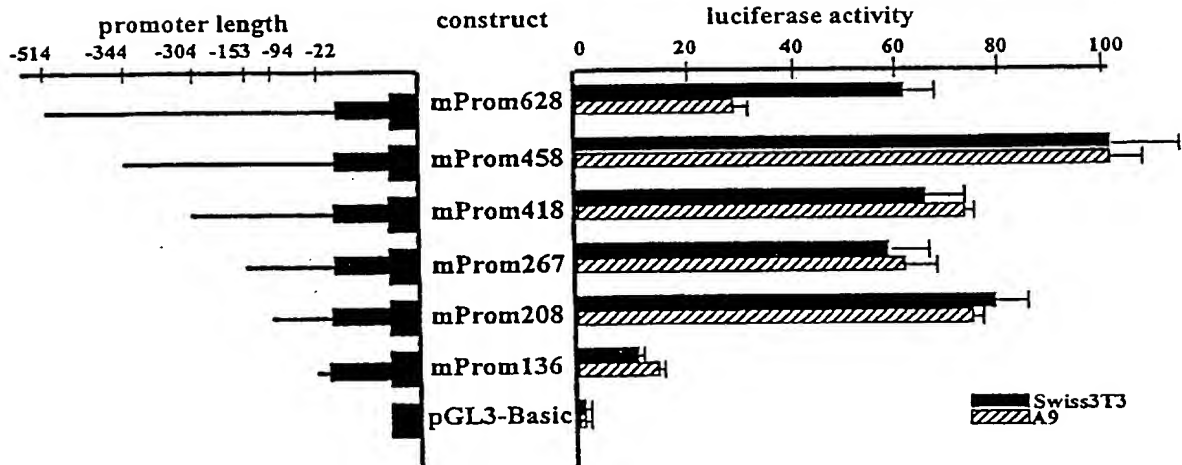
-48 cggtcggtcgccgctccctttataagccgactcgccggcagcgcaccgg
hProm111
PEA2/PEBP2 GAGA TBP/TFIID

gttgcggagggtgggcctgggaggggtggtggccatttttgtctaaccc
template
taactgagaagggcgta hProm

Fig. 4A

Fig. 4B

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*Fig. 5A**Fig. 5B*

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Oligo's Used: human

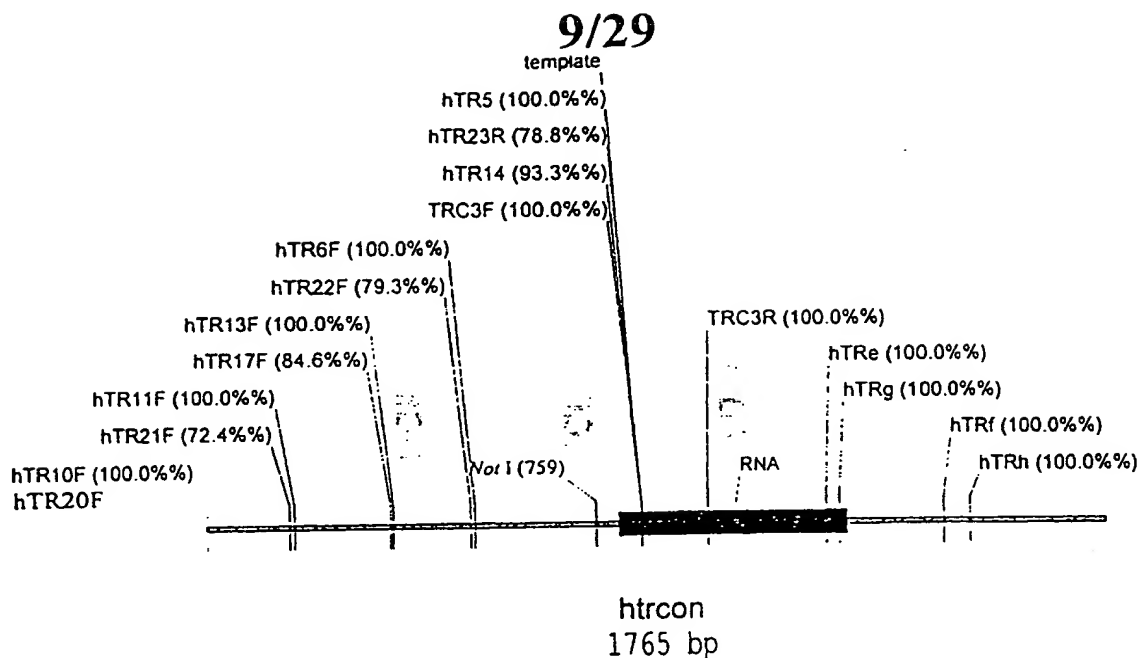
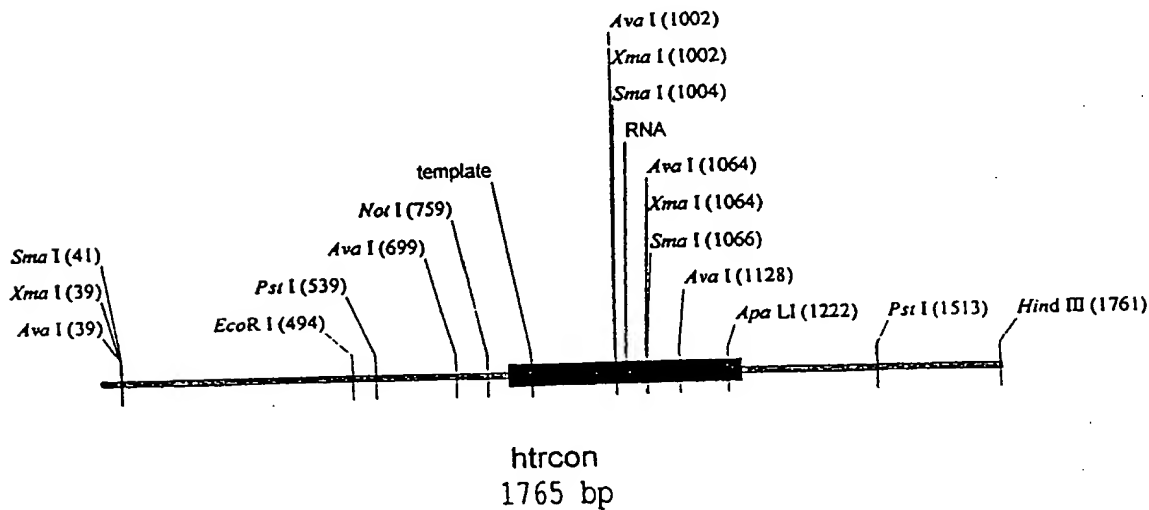
Name	Sequence	Comments
hTR5	TACGCCCTTCTCAGTTAGGGTTAG	
hTR14	GGATCCTACGCCCTTCTCAGTTAGGGTTAG	hTR5 with BamHI site
hTR13F	ACTGAGCCGAGACAAGATTC	
hTR17F	GGATCCACTGAGCCGAGACAAGATTC	hTR13F with BamHI site
hTR10F	AGCTACTCAGGAGGCTGAGA	
hTR20F	GCGCTCGAGAGCTACTCAGGAGGCTGAGA	hTR10F with XhoI site plus gcg clamp
hTR11F	CATCAAGACACAGCACTACT	
hTR21F	GCGCTCGAGCATCAAGACACAGCACTACT	hTR11F with XhoI site plus gcg clamp
hTR6F	GTCTGGTCTGCAGAGGATAG	
hTR22F	GCGCTCGAGGTCTGGTCTGCAGAGGATAG	hTR6F with Xho site plus gcg clamp
hTR5	TACGCCCTTCTCAGTTAGGGTTAG	
hTR23R	CGCAAGCTTTACGCCCTTCTCAGTTAGGGTTAG	hTR5 with HindIII site plus cgc clamp
hTRe	CTGAGCTGTGGGACGTGCAC	
hTRf	AGACGGGAGAACCCACGCAG	
hTRg	CTCGGCTCACACATGCAGTT	
hTRh	TCTGCAGAGCAGGAAGTAAGT	
TRC3F	CTAACCCCTAACTGAGAAGGGCGTA	
TRC3R	GGCGAACGGGCCAGCAGCTGACATT	

Oligo's Used: Mouse

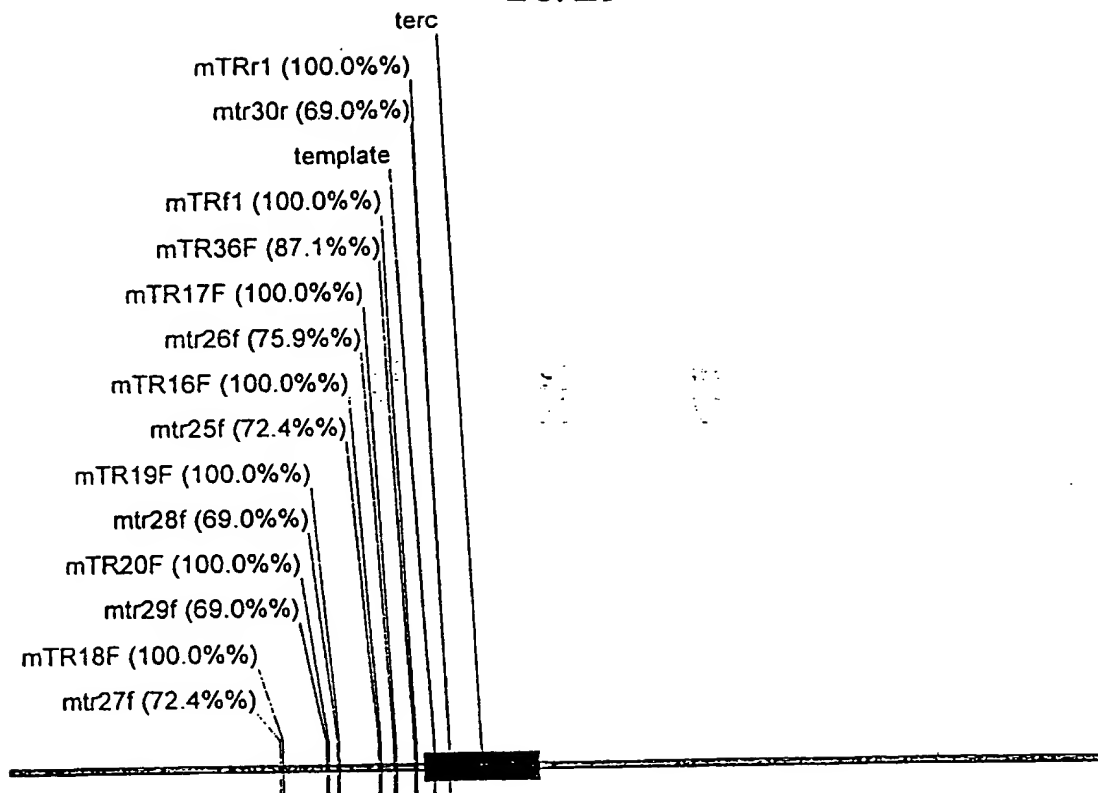
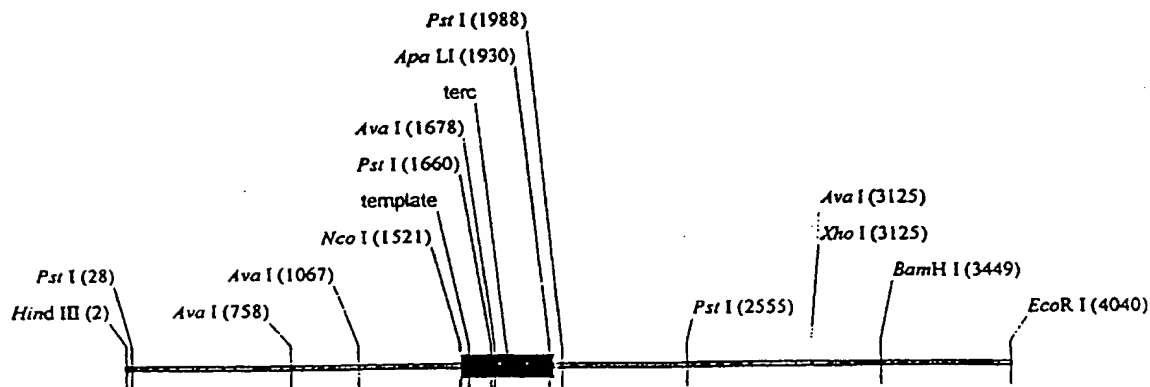
Name	Sequence	Comments
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mtr25f	GCGCTCGAGGTGTCTCACAGCAAGAAACA	This is mtr16f with XhoI site plus gcg clamp
mTR17F	GTGACTGGCTAGGAAGAGTG	
mtr26f	GCGCTCGAGGTGACTGGCTAGGAAGAGTG	This is mtr17f with XhoI site plus gcg clamp
mTR18F	TGTGACCTTGAAGTACAGAC	
mtr27f	GCGCTCGAGTGTGACCTTGAAGTACAGAC	This is mtr18f with XhoI site plus gcg clamp
mTR19F	GGACTGGGTTGAAGGTGGAA	
mtr28f	GCGCTCGAGGGACTGGGTTGAAGGTGGAA	This is mtr19f with XhoI site plus gcg clamp
mTR20F	TGCGCCACTTTTCCCCACTT	
mtr29f	GCGCTCGAGTGCGCCACTTTTCCCCACTT	This is mtr20f with XhoI site plus gcg clamp
mTRr1	CCGCTGGAAGTCAGCGAGAA	
mtr30r	CGCAAGCTTCCGCTGGAAGTCAGCGAGAA	This is mTRr1 with HindIII site plus cgc clamp
mTR36F	GCGCTCGAGTCGACCAATCAGCGCGGCCAT	This is Xho I site PCR primer plus gcg clamp
mTRr1	CCGCTGGAAGTCAGCGAGAA	
mTRf1	TCGACCAATCAGCGCGGCCAT	

Fig. 6

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**Fig. 7A****Fig. 7B**

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tercCon
4044 bp**Fig. 8A**tercCon
4044 bp**Fig. 8B**

005280" 29210960

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TTGTGACCTTGAACACAGACCTCCTGCTCAGCCTCCTACAAGCTGGGATTATAGGCTCGGTCAGCTACCCCTTGAAA
TTCTTTTCTTCTGGAACACAGTACCTGGTTGGCCATGCACACTACAAGAGATCCGCCCTGCCCTTCTGTCAAATTCCTGGA
ATTAAAGATTTCGCCCACTTTCCCCCACTTCCACCCCGGCTGTGGAGTGGACTGGGTGAAGGTGGAATTTTCTTTT
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AAAAATGTGCGTGCGTGCTGT
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AAGTGTGGGCTCGACCAATCAGCGCGCGCCATGGGGTATTAAAGTTCGAGGGCGGCTAGGCCCTCGGCACCTAACCCCTG
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Fig. 9

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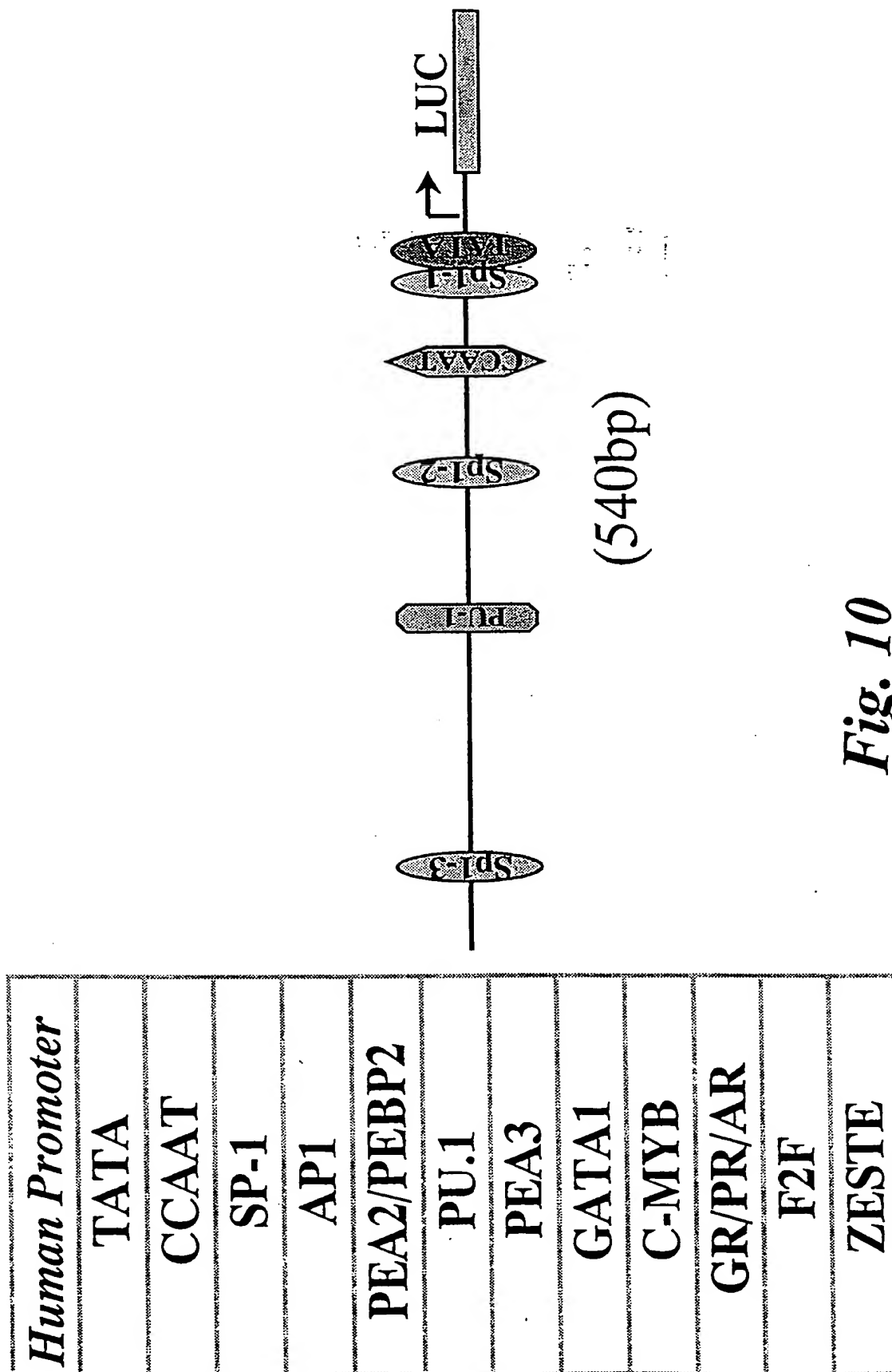


Fig. 10

-107Sp1-2
2923 (wt) AGCCCGCCGAGAGAGTGACTCTCAGAGAGCCGCGAGAGTCAGCTTGGCCAA CCAAT-box⁻⁵⁵

-54
2923 (wt) TCCGTGGGTCCGGCCGCTCCCTTTATAAGCCGACTCGCCCCGGCAGCGCACC⁻¹
+1
GGGTTCCGGAGGGTGGGCTGGGAGGGGTGGTGGCCATTTTGTCTAACC⁺⁵²

Sp1-1

RCE Sp1-4

+53
CTAACTGAGAAGGGCGTA⁺⁶⁹

Fig. 11

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Fig. 12

Oligonucleotide	Position	Sequence*	Purpose
h11 ^a	-2 to +36	CCGGTTGCGAGGGTGGGCTGGAGGGGTGGTGCC	RCE(+12, +16 and +30, +34) and Sp1.4 binding site
h111 ^a	"	CCGGTTGCGGAAATGGGCTGGAGGGGTGGTGCC	RCE1 mutation from ggg to aaa (+11/+13)
h112 ^a	"	CCGGTTGCGGAGGGTGGGCTGGGTAAAGGTGGTGCC	Sp1.4 mutation from agg to taa (+24/+26)
h113 ^a	"	CCGGTTGCGGAAATGGGCTGGGTAAAGGTGGTGCC	Mutant of both RCE1 and Sp1.4 binding site(+11/+13, +24/+26)
h11c ^a	-2 to +23	CCGGTTGCGGAGGGTGGGCTGGG	RCE1 binding site
h11d ^a	+14 to +36	GCCTGGGAGGGTGGTGCC	Sp1.4 or RCE2 binding site
h111a ^{a,b}	"	CCGGTTGCGGAAATGGGCTGGG	RCE1 mutation from ggg to aaa(+11/+13)
h112b ^{a,b}	+15 to +36	GGGCTGGGTAAAGGTGGTGCC	Sp1.4 mutation from agg to taa
h112C ^{a,b}	"	GGGCTGGGTAAAGGTAAATGGCC	Sp1.4 and RCE2 mutant from aggggtgg to taaggtaa(+24/+26, +30/+31)
h11e ^a	"	GGGCTGGGAGGGGTAAATGGCC	RCE2 mutant from gg to (+30/+31)
h10 ^a	-63 to -42	CTTGGCCAAATCCGTCGGTCCG	h10 bootprinting region containing CCAAT binding site
h101 ^a	-63 to -42	CTTGGAGTCTCCGTCGGTCCG	CCAAT motif mutation from ccaa to agtc, (-58/-55)
h10m11 ^b	-74 to -45	GCGAGAGTCAGCTTGGAGTCTCCGTCGG	CCAAT motif mutation from ccaa to agtc, (-58/-55)
h10m2 ^{a,b}	-63 to -42	CTTGGCCAAATCCGTCGATGGTCCG	h10 mutation from gtgc to tgal, (-51/-47)
h9 ^a	-44 to -17	CGGCGCCGCTCCCTTTATAGCCGACT	h9 bootprinting region containing SP1.1 binding site
h91 ^a	-44 to -21	CTTAGCCGCTCCCTTTATAGCC	h9 mutation from ggog to ttac, (-43/-40)
h910 ^b	-53 to -29	CCGTGCGGTCTTACGCGCTCCC	h9 mutation from ggog to ttac, (-43/-40)
h911 ^a	-44 to -21	CGGCTAAACTCCCTTTATAGCC	h9 mutation from ggog to taaa, (-39/-36)
h92 ^a	-44 to -21	CGGCGCCATAGCCTTTATAGCC	h9 mutation from gctc to alag, (-36/-33)
h921 ^a	-44 to -21	CGGCGCCGCTCATGCTATAGCC	h9 mutation from cctt to algc, (-32/-29)
h93 ^a	-44 to -21	CGGCGCCGCTCCCTTCGACAGCC	h9 mutation from tata to cgac, (-28/-25)
h930 ^b	-38 to -14	CCGCTCCCTTCGACAGCGGACTCGC	h9 mutation from tata to cgac, (-28/-25)
h4 ^a	-110 to -91	ACCAGCCGCCCGAGAGAGT	h4 bootprinting region containing SP1.2 binding site
h41m ^a	-110 to -91	ACCAGCCCGAACGAGAGAGT	SP1.2 mutation from cc to aa, (-101/-100)
h5 ^a	-471 to -452	GAAAAAGGGCAGGGTTGGA	SP1.3 binding site
h5m ^a	-471 to -452	GAAAAAGGTTACGGGTTGGA	SP1.3 mutation from gg to tt, (-463/-462)

* Nucleotides corresponding to promoter sequences are given in uppercase letters from 5' end to 3' end; specific nucleotides mentioned in Purpose column are underlined. Highlight indicate mutagenesis nucleotide.

^a Complementary lowerstrand sequence for EMSA not shown.

^b For PCR-directed in vitro mutagenesis, complementary lowstrand sequence not shown.

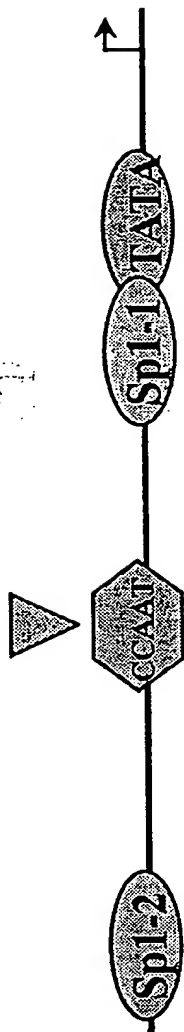


Fig. 13

Probes: Sp1-2 Sp1-1

Sp1-2

Spl-1

I
+Sp1
+Sp3
+Ets2
+Ap2

$$\begin{array}{l} +s p_1 \\ +s p_3 \\ +\Delta p_2 \end{array}$$

Antibody: —

sp¹ → sp³ → sp³ → N →

↑
[E]

1 2 3 4 5 6 7 8 9

↓

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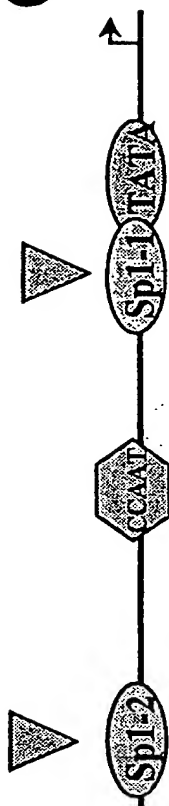
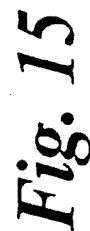


Fig. 14



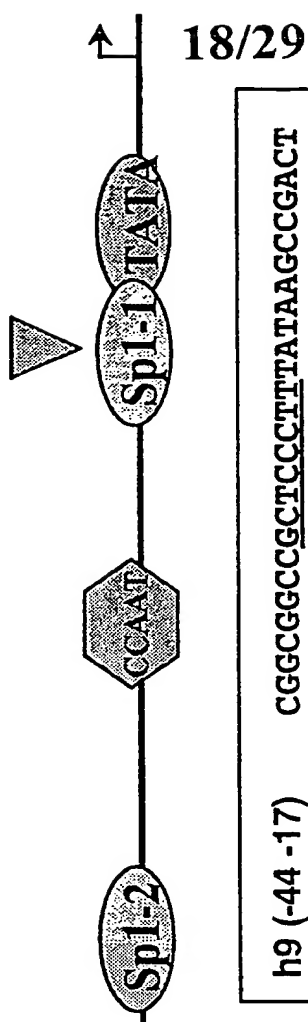
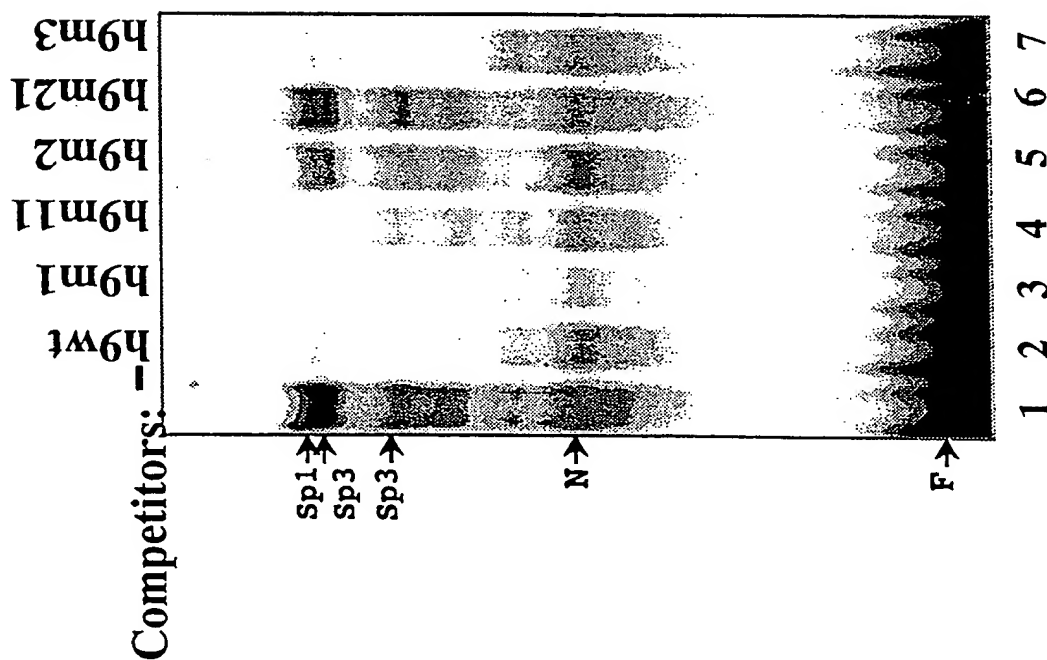


Fig. 16

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- Gel shift assays identify DNA/protein binding activity
- Do mutation of these binding sites influence the promoter activity?

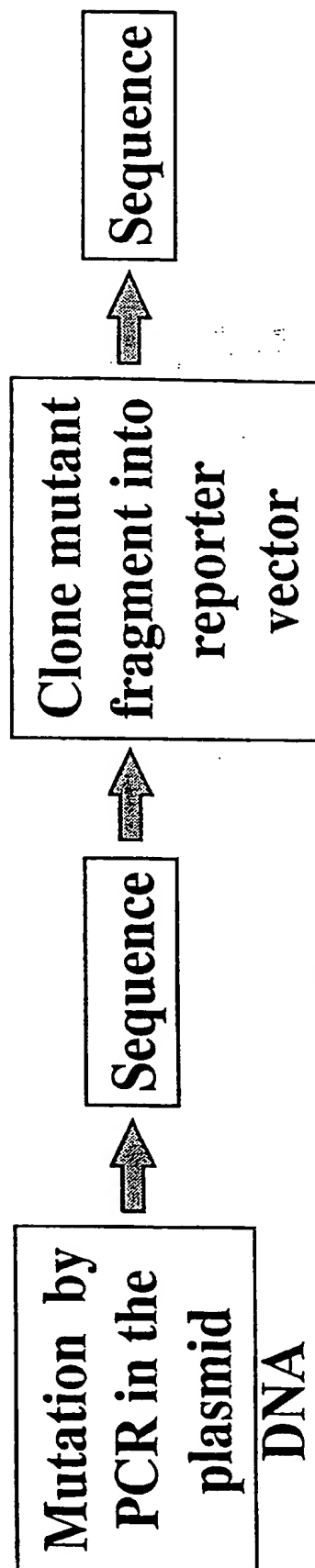


Fig. 17

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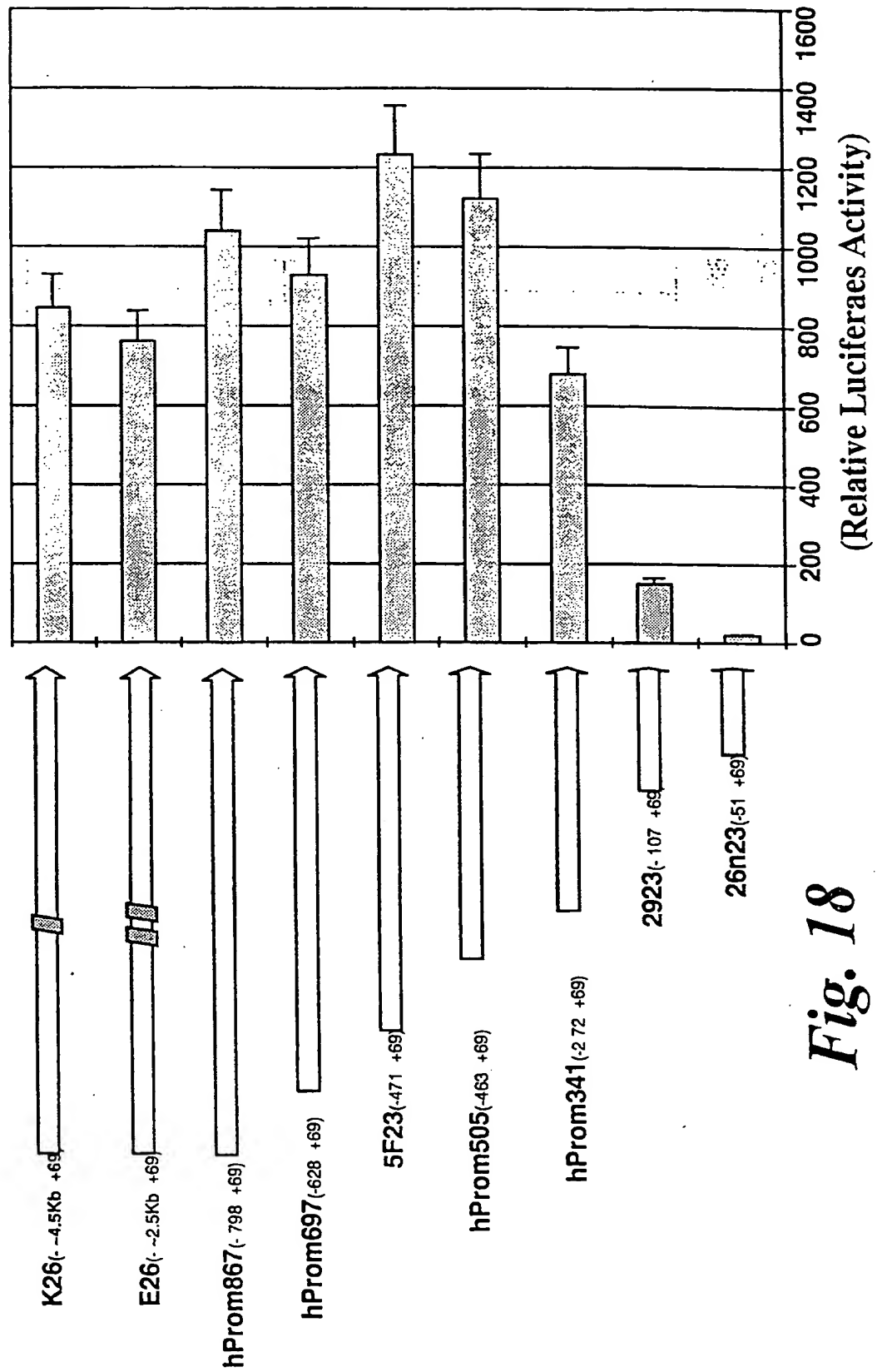


Fig. 18

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2923(-107 +69) -107 -55
 29m23(mSp1.2) AGCCCCCGAGAGAGTGACTCTCACGAGAGCCGCGAGAGTCAGCTTGGCCAA
 1011 -----AA-----
 29m292(mSp1-1,2) -----AA-----
 29m921(mSp1-1,2) -----AA-----

 2923(-107 +69) -54 -1
 102 TCCGTGCGGTCGGCGGCCGCTCCCTTTATAAGCCGACTCGCCCGGCAGCGCACC
 910 ---TGAT-----
 911 -----TTAC-----
 911 -----TAAA-----
 92(mSp1.1) -----ATAG-----
 29m292(mSp1-1,2) -----ATAG-----
 921(mSp1.1) -----ATGC-----
 29m921(mSp1-1,2) -----ATGC-----
 930(mTATA) -----CGAC-----
 26n23(-51 +69) -----

 26n23(-51 +69) +1 +53
 29111(mRCE) GGGTTGCGGAGGGTGGGCCTGGGAGGGGTGGTGGCCATTTTTTGTCTAACC
 29112(mSp1.4) -----AAA-----
 111(Sp1.4)* -----TAA-----
 112(RCE)* ---//---AAA-----TAA-----
 113(mSp1+mRCE)* ---//---AAA-----TAA-----
 115* ---//---AAA-----TAA-AA-----
 114* ---//---AAA-----TAA-AA-----

 +54 +69
 CTAAGTGAAGGGCGTA

* Double mutagenesis by using pLh29m292 as template.

Fig. 19

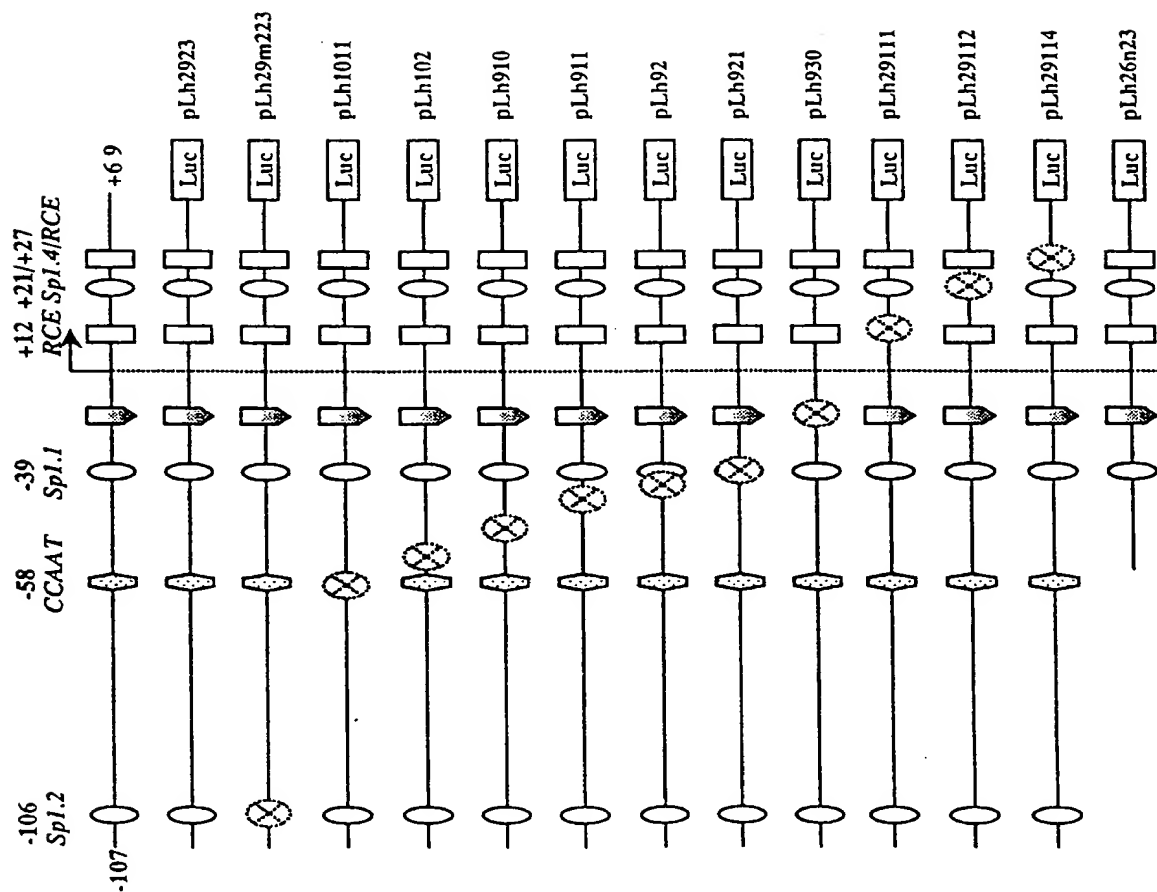


Fig. 20

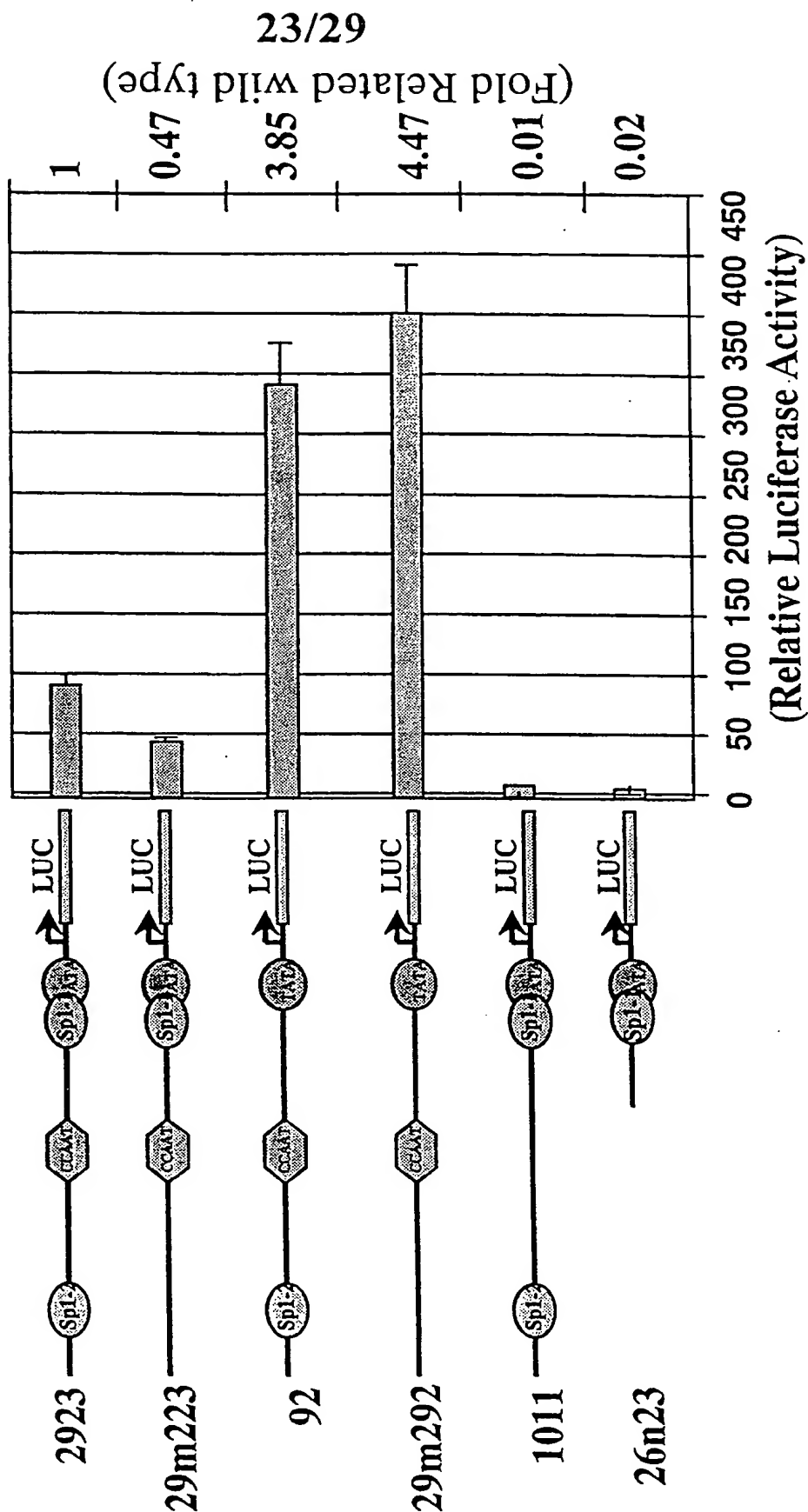


Fig. 21

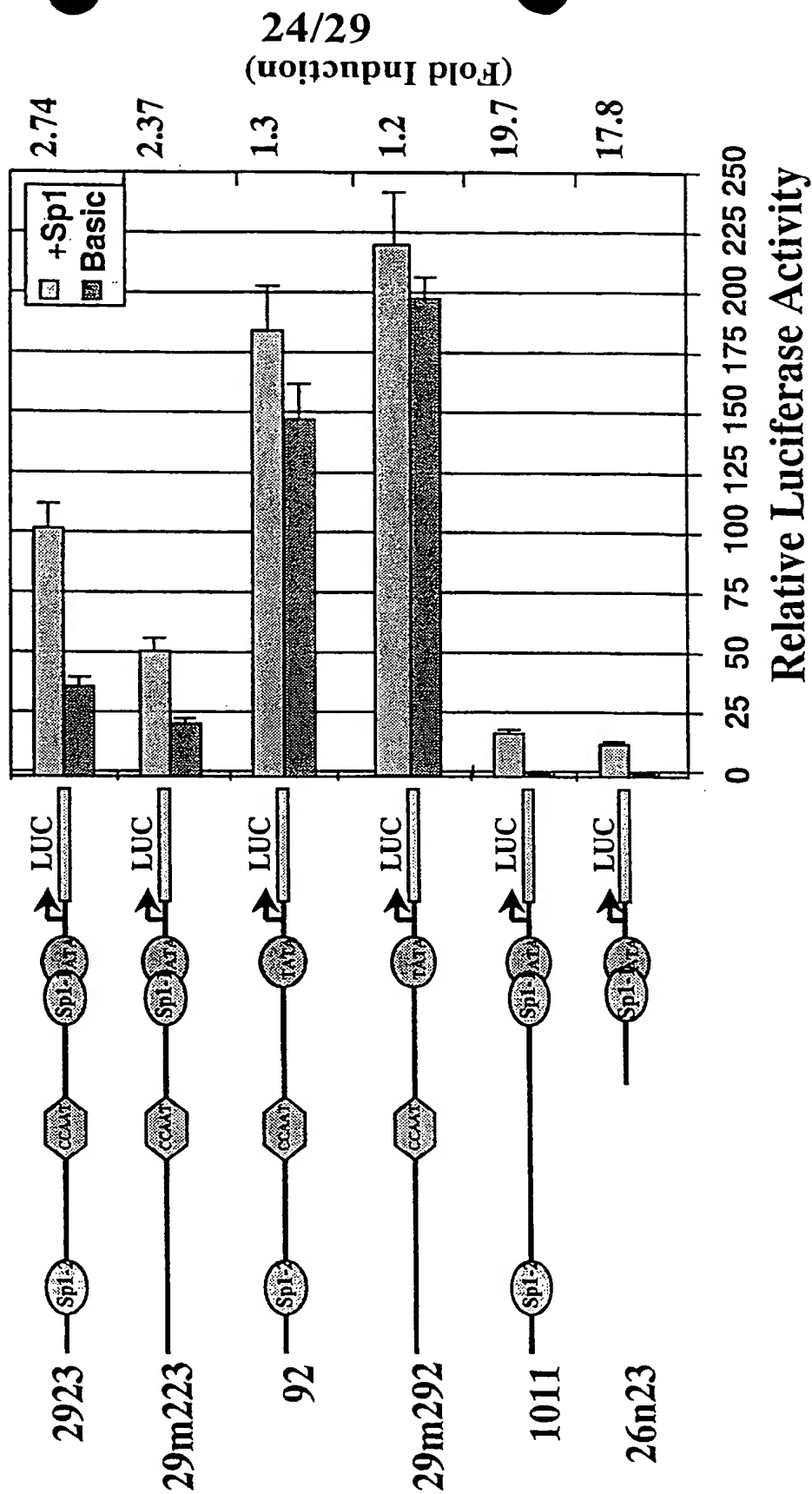


Fig. 22

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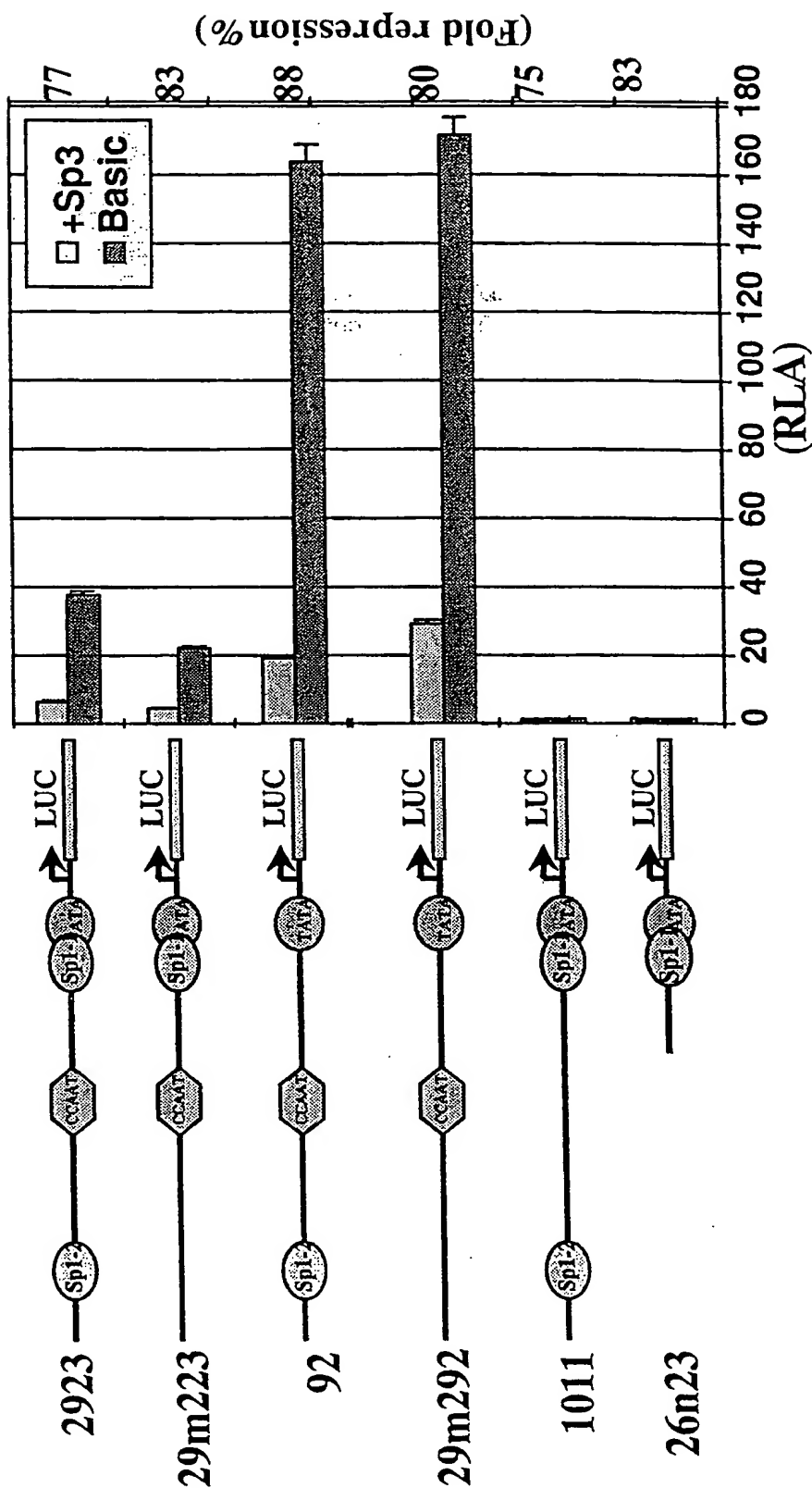
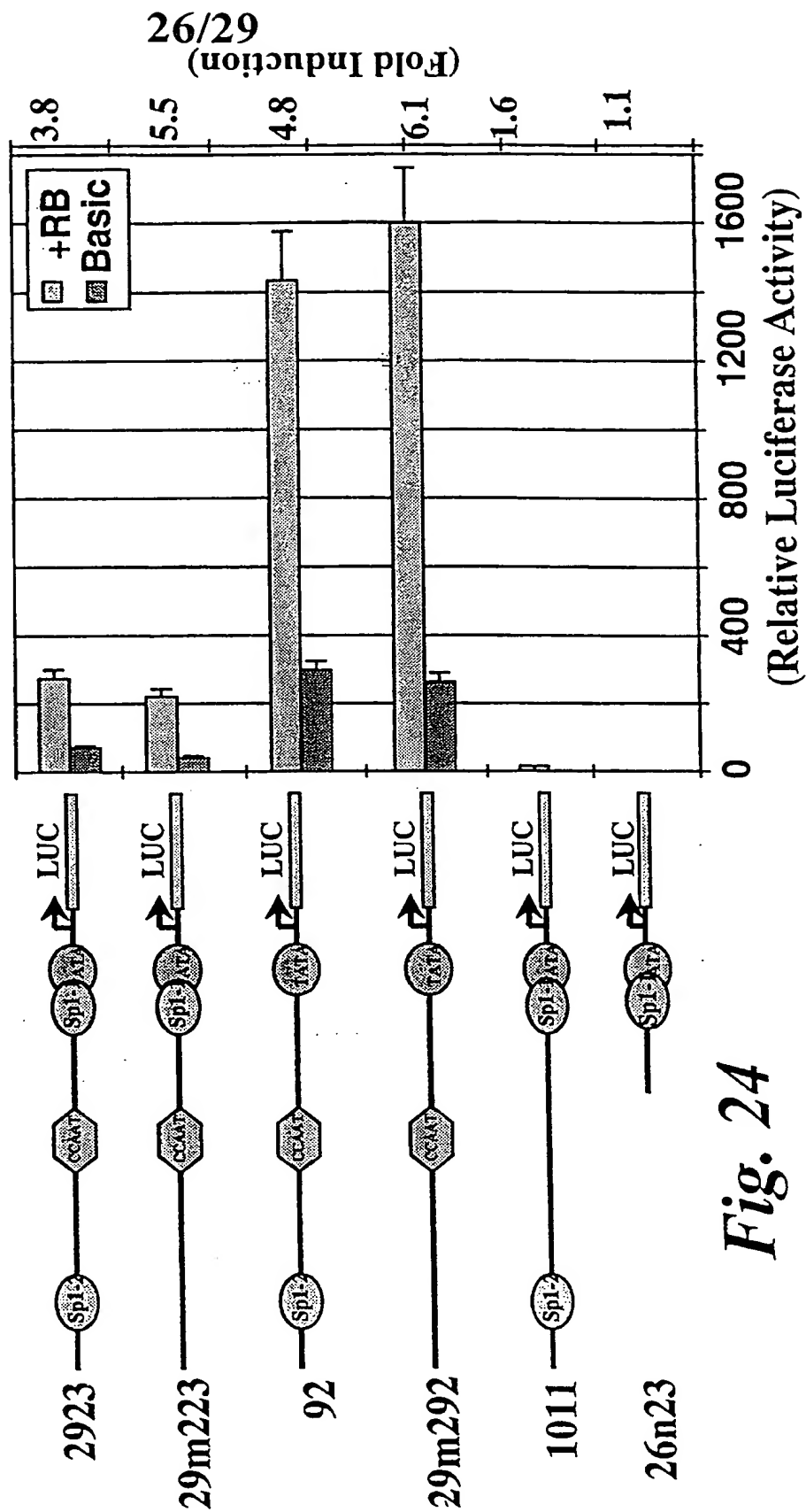
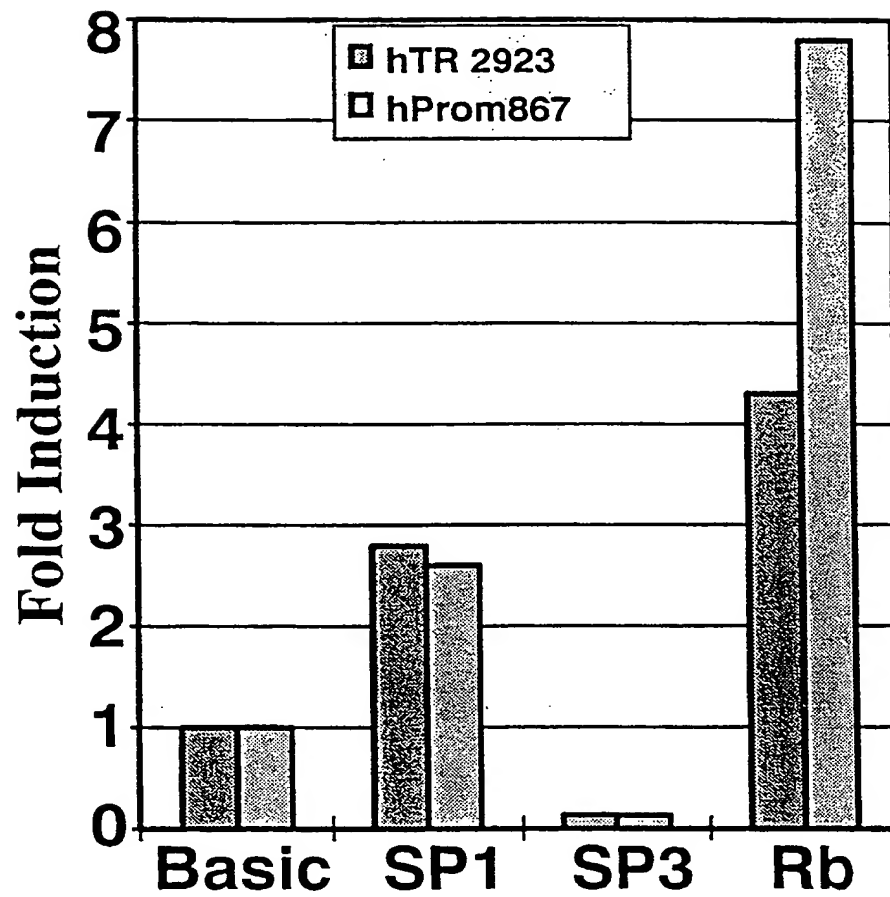


Fig. 23

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*Fig. 25*

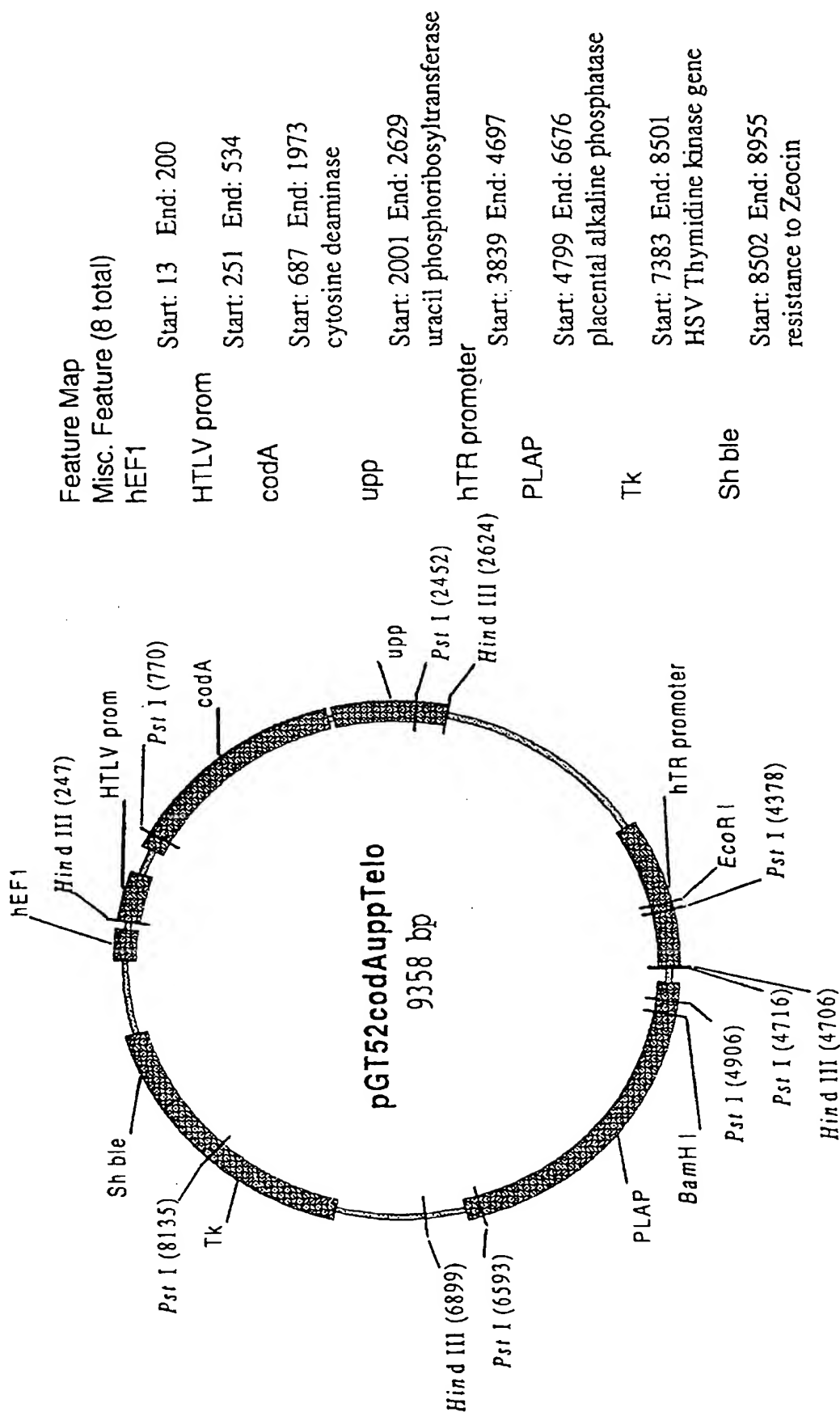


Fig. 26

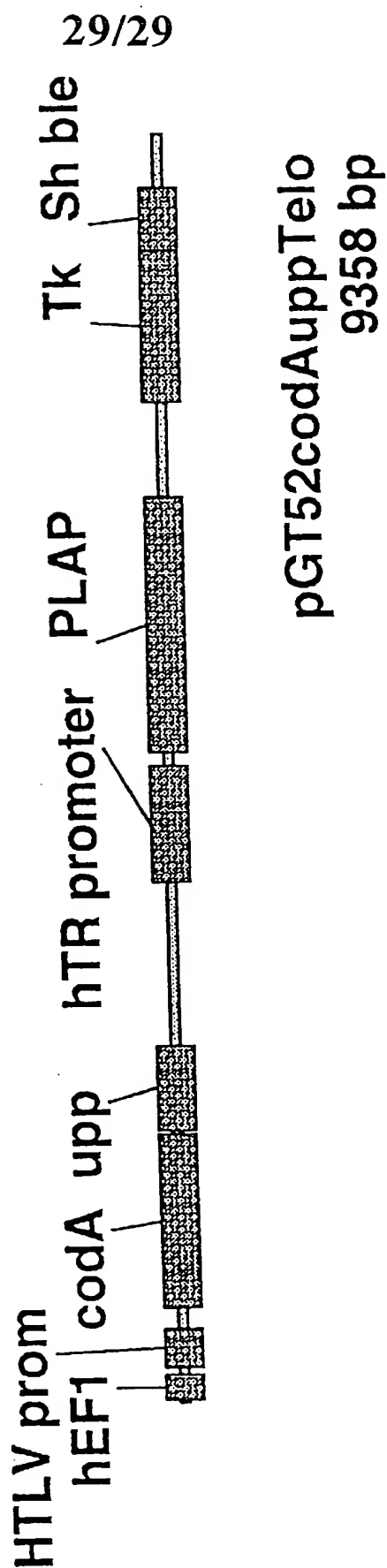


Fig. 27